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The ratio of S-adenosylmethione and S-adenosyl-homocysteine is increased in the brains of newborn rats in a model of valproic acid teratogenicity

Letter to the Editor

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The antiepileptic drug valproic acid (VPA) has a high teratogenic potential leading to major fetal malformations such as neurotubular defects. In addition to that children of women with epilepsy, exposed to valproate (VPA) in utero, show a significant decrease of intelligence and attention (Meador et al. 2009). Until now the mechanisms of the teratogenic effects of VPA are unclear.

In their article “Multiple point action mechanism of valproic acid-teratogenicity alleviated by folic acid, vitamin C and N-acetylcysteine in chicken embryo model” Hsieh et al describe several possible mechanisms of VPA-induced teratogenicity, such as lowering the levels of homocysteine, folate, glutathione and induction of

reactive oxygen species. In addition, they showed inhibition of two important enzymes of the homocysteine metabolism, that is dihydrofolate reductase and methylene folate reductase by VPA.

In a rodent model of valproic acid teratogenicity we have found dose-dependent changes of S-adenosylmethione (SAM) and S-adenosyl-homocysteine (SAH) concentrations in the brains of newborn pups. Our results complement the data presented by Hsieh et al, who have not included SAM and SAH in their analysis. SAM and SAH occupy a central position in the homocysteine metabolism. SAM is an essential methyl donor to maintain normal methylation of DNA, RNA, proteins, and several other molecules for normal cell function and viability (Chiang et al. 1996; Ulrey et al. 2005). The degradation product of SAM is SAH, which is reversibly hydrolyzed to homocysteine. The transsulfuration of homocysteine finally leads to the production of glutathione. As an alternative to transsulfuration, Hcys can be recycled by remethylation via methionine synthase. This requires vitamin B12 and folate as co-factors.

To induce VPA teratogenicity, we diluted valproate into the drinking water of female Wistar rats starting 7days before conception and during pregnancy. With drug concentrations of 3.3mg/ml (n=6), and 6.6 mg/ml (n=6), daily valproate dosages of about 470mg/kg (medium dosage (MD), and 720 mg/kg (high dosage (HD)) were reached. We have previously shown that this intrauterine VPA exposure leads to changes in activity and memory and changes in volume of different brain areas (Frisch et al. 2009).

Newborn offspring (p1) with intrauterine VPA exposure and controls were perfused with a PBS – buffer, and brains were removed and stored at -80C. In brain lysates we performed simultaneous determination of SAM and SAH by using stable isotope dilution tandem mass spectrometry as previously published (Smith et al. 2006).

There was a significant, dose-depended increase in the SAM/SAH ratio in brain tissues of VPA exposed animals.

	Mean ± SD	F	p	Bonferoni Controls	MD	HD
Controls	7.88	9.56	0.002	-	0.162	0.002
MD	10.44			0.162	-	0.113
HD	13.24			0.002	0.113	-

Table 1: SAM/SAH ratio in brain lysates of newborn pups (P1)

These results further corroborate the findings of Hsieh et al and the assumption that disruption of folate and homocysteine metabolism is a possible teratogenic mechanism of VPA treatment. Because SAM and SAH are the substrate and product of essential methyltransferase reactions, and because SAH is an effective antagonist of transmethylation enzymes, the ratio of SAM/SAH is used as an indicator of the cellular methylation potential (Cantoni 1985; Hoffman et al. 1979). An increased methylation potential will influence numerous reactions involved in cellular metabolism, in particular DNA methylation (Detich et al. 2003; Rodenhiser and Mann 2006), which is considered to be essential in neuronal cell differentiation (Singh et al.

2009). The increase of the SAM/SAH ratio also leads to up-regulation of cystathionine beta-synthase (CBS) activity (Selhub and Miller 1992), which increases the flux of homocysteine through the transsulfuration pathway. CBS is additionally upregulated by production of reactive oxygen species (Singh et al. 2007), as measured by Hsieh et al. Both factors explain the observed decrease in homocysteine levels.

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